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University of Massachusetts Amherst
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14. ABSTRACT
Feedback regulated drug delivery vehicles are capable of utilizing the physiological response as a signal to modulate drug release (i.e., trigger, slow down, or stop drug release) from the carrier. Such vehicles hold great promise for effective drug delivery, especially when therapeutic drugs exhibit lethal consequences at high concentrations. An area that requires particular attention from feedback regulated drug delivery involves release of antidotes in response to opioid overdose. For example, overdose of morphine causes hypoventilation, an inadequate

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a. REPORT	b. ABSTRACT	c. THIS PAGE			Sankaran Thayumanavan
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Report Title

Feedback Drug Delivery Vehicles

ABSTRACT

Feedback regulated drug delivery vehicles are capable of utilizing the physiological response as a signal to modulate drug release (i.e., trigger, slow down, or stop drug release) from the carrier. Such vehicles hold great promise for effective drug delivery, especially when therapeutic drugs exhibit lethal consequences at high concentrations. An area that requires particular attention from feedback regulated drug delivery involves release of antidotes in response to opioid overdose. For example, overdose of morphine causes hypoventilation, an inadequate ventilation to perform gas exchanges in lungs leading to increased CO2 concentration in the blood. Therefore, we were interested in taking advantage of CO2 as a toxicity marker, to design a polymeric-hydrogel-based delivery vehicle which is capable of releasing antidotes in response to biomarker. In this project, we specifically aimed at the design and synthesis of polymer hydrogels and nanogels for morphine and naloxone release in response to biomarker, CO2.

Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Received		Paper
12/13/2011	1.00	Ja-Hyoung Ryu, Raghunath Roy, Judy Ventura, S. Thayumanavan. Redox-Sensitive Disassembly of Amphiphilic Copolymer Based Micelles, Langmuir, (05 2010): 0. doi: 10.1021/la904437u
12/13/2011	5.00	Malar A. Azagarsamy, Punidha Sokkalingam, S. Thayumanavan. Enzyme-Triggered Disassembly of Dendrimer-Based Amphiphilic Nanocontainers, Journal of the American Chemical Society, (10 2009): 0. doi: 10.1021/ja906162u
12/13/2011	2.00	Siriporn Jiwpanich, Ja-Hyoung Ryu, Sean Bickerton, S. Thayumanavan. Noncovalent Encapsulation Stabilities in Supramolecular Nanoassemblies, Journal of the American Chemical Society, (08 2010): 0. doi: 10.1021/ja105059g
12/20/2011	3.00	Ja-Hyoung Ryu, Reuben T. Chacko, Siriporn Jiwpanich, Sean Bickerton, R. Prakash Babu, S. Thayumanavan. Self-Cross-Linked Polymer Nanogels: A Versatile Nanoscopic Drug Delivery Platform, Journal of the American Chemical Society, (12 2010): 0. doi: 10.1021/ja1069932
12/20/2011	4.00	Reuben Chacko, Sean Bickerton, S. Thayumanavan, Ja-Hyoung Ryu, Siriporn Jiwpanich. Surface-Functionalizable Polymer Nanogels with Facile Hydrophobic Guest Encapsulation Capabilities, Journal of the American Chemical Society, (06 2010): 0. doi: 10.1021/ja102316a
TOTAL:	**5**	

Number of Papers published in peer-reviewed journals:

(b) Papers published in non-peer-reviewed journals (N/A for none)

Received Paper

TOTAL:

Number of Papers published in non peer-reviewed journals:

(c) Presentations

Number of Presentations: 0.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received Paper

TOTAL:

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):

(d) Manuscripts

Received Paper

TOTAL:

Number of Manuscripts:

Books

Received Paper

TOTAL:

Patents Submitted

Patents Awarded

Awards

Graduate Students

NAME	PERCENT_SUPPORTED	Discipline
Diego Amado Torres	0.03	
Reuben Chacko	0.15	
Nagamani Chikkannagari	0.11	
Jack Fuller	0.02	
Daniella Gonazalez	0.01	
Jing Guo	0.03	
Siriporn Jiwpanich	0.70	
Akamol Klaikherd	0.11	
Michael Lartey	0.67	
Longyu Li	0.05	
Oyuntuya Munkhbat	0.03	
Gladys Murage	0.07	
Kishore Raghupathi	0.03	
Krishna Raghupathi	0.06	
Rajasekhar Reddy Rami Reddy	0.07	
Elamprakash Savariar	0.11	
Judy Ventura	0.07	
Feng Wang	0.05	
Volkan Yesilyurt	0.02	
Jiaming Zhang	0.29	
FTE Equivalent:	**2.68**	
Total Number:	**20**	

Names of Post Doctorates

NAME	PERCENT_SUPPORTED
Subhadeep Basu	0.07
Shreedhar Bhat	0.26
Byron Collins	0.18
Antara Dasgupta	0.18
Deepak Dharmangadan	0.11
Arisa Jaiyu	0.19
Jayaprakash Pagdala	0.25
Anupat Potisatityuenyong	0.33
Prakash Rajendran	0.53
Thirumoorthi Ramalingam	0.09
Ja-Hyoung Ryu	0.65
Sunita Satav	0.46
Narayana Murthy Sekar	0.44
Punidha Sokkalingam	0.35
Ayyagari Subrahmanyam	0.03
FTE Equivalent:	**4.12**
Total Number:	**15**

Names of Faculty Supported

NAME	PERCENT_SUPPORTED	National Academy Member
Kothandam Krishnamoorthy	0.37	
Sankaran Thayumanavan	0.07	
FTE Equivalent:	**0.44**	
Total Number:	**2**	

Names of Under Graduate students supported

NAME	PERCENT SUPPORTED	Discipline
Tal Aharon	0.06	Chemistry
David Waterman	0.04	Chemistry
Jennifer Wilcox	0.05	Chemistry
FTE Equivalent:	**0.15**	
Total Number:	**3**	

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period:	1.00
The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:	1.00
The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:	1.00
Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):	1.00
Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:	0.00
The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense	0.00
The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields:	0.00

Names of Personnel receiving masters degrees

NAME
Total Number:

Names of personnel receiving PHDs

NAME
Siriporn Jiwpanich
Elamprakash Savariar
Nagamani Chikkannagari
Akamol Klaikherd
Michael Lartey
Volkan Yesilyurt
Total Number: 6

Names of other research staff

NAME	PERCENT SUPPORTED
Sean Bickerton	0.25
Aidan McKenna	0.01
FTE Equivalent:	**0.26**
Total Number:	**2**

Sub Contractors (DD882)

1 a. Stanford University

1 b. Office of Sponsor Research
Board of Trustees of the Leland Stanforc
Stanford CA 943054100

Sub Contractor Numbers (c): 08-004439 B 03
Patent Clause Number (d-1): 52.227-11
Patent Date (d-2): 6/1/1997 12:00:00AM
Work Description (e): Dr. Angst is an expert anesthesiologist whose primary role in this project is as clinician consultant.
Sub Contract Award Date (f-1): 8/1/2007 12:00:00AM
Sub Contract Est Completion Date(f-2): 10/30/2011 12:00:00AM

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1 a. Stanford University

1 b. Mail Stop 4125, Room 110
651 Serra Street
Stanford CA 943054125

Sub Contractor Numbers (c): 08-004439 B 03
Patent Clause Number (d-1): 52.227-11
Patent Date (d-2): 6/1/1997 12:00:00AM
Work Description (e): Dr. Angst is an expert anesthesiologist whose primary role in this project is as clinician consultant.
Sub Contract Award Date (f-1): 8/1/2007 12:00:00AM
Sub Contract Est Completion Date(f-2): 10/30/2011 12:00:00AM

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1 a. Oregon Health and Science University

1 b. 3181 S.W. Sam Jackson Park Rd.
Portland OR 97239-3098

Sub Contractor Numbers (c): 08-004439 A
Patent Clause Number (d-1): 52.227-11
Patent Date (d-2): 6/1/1997 12:00:00AM
Work Description (e): The OHSU component of this project centered on the refining, improving and up-scaling of the deli
Sub Contract Award Date (f-1): 8/1/2007 12:00:00AM
Sub Contract Est Completion Date(f-2): 10/30/2011 12:00:00AM

Inventions (DD882)

Scientific Progress

See Attachment

Technology Transfer

Feedback Regulated Drug Delivery Vehicles (W911NF0710462)

Submitted by:

S. Thayumanavan,

University of Massachusetts Amherst

Final Report

Table of Contents:

Feedback Regulated Drug Delivery Vehicles
(W911NF0710462)

Submitted by: S. Thayumanavan, University of Massachusetts Amherst

Feedback regulated drug delivery vehicles are capable of utilizing the physiological response as a signal to modulate drug release (i.e., trigger, slow down, or stop drug release) from the carrier. Such vehicles hold great promise for effective drug delivery, especially when therapeutic drugs exhibit lethal consequences at high concentrations. An area that requires particular attention from feedback regulated drug delivery involves release of antidotes in response to opioid overdose. For example, overdose of morphine causes hypoventilation, an inadequate ventilation to perform gas exchanges in lungs leading to increased CO_2 concentration in the blood. Therefore, we were interested in taking advantage of CO_2 as a toxicity marker, to design a polymeric-hydrogel-based delivery vehicle which is capable of releasing antidotes in response to biomarker. In this project, we specifically aimed at the design and synthesis of polymer hydrogels and nanogels for morphine and naloxone release in response to biomarker, CO_2.

2007-08

Design and Synthesis of Polymeric Hydrogels for Morphine Release

We have synthesized tens of gels to identify the ideal candidate for controlled release of morphine. We detail here the more successful candidates for this purpose. We first prepared hydrogels based on acrylic acid monomer and ethylene glycol dimethacrylate cross-linker. This gel provided a slow decrease in the release of morphine with time for about 8 h (Figure 1). Next, we prepared same kind of gels by replacing acrylic acid with sodium acrylate. We found that changing the acrylic acid with sodium salt resulted in burst release. The gel released its most of morphine contents in about 1 h (Figure 2a).

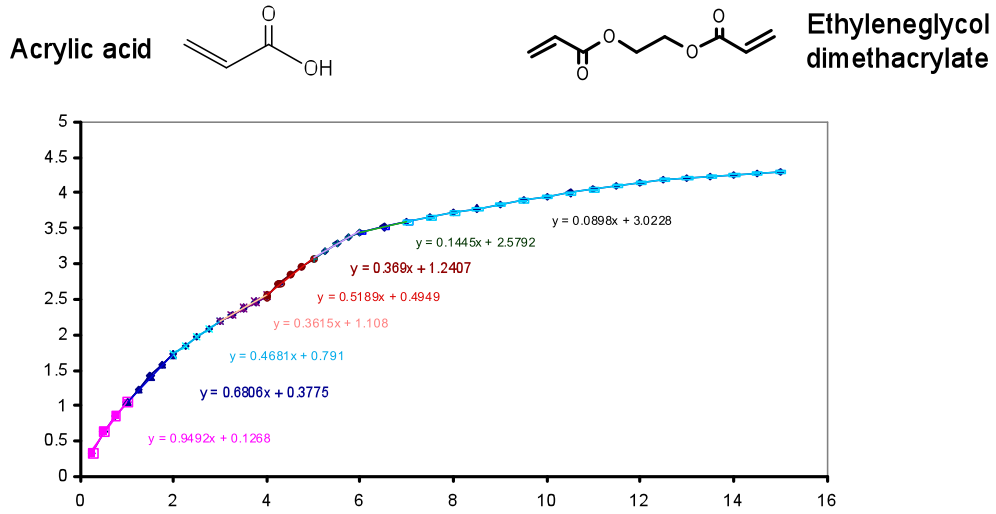

Figure 1. Release profile of Morphine.

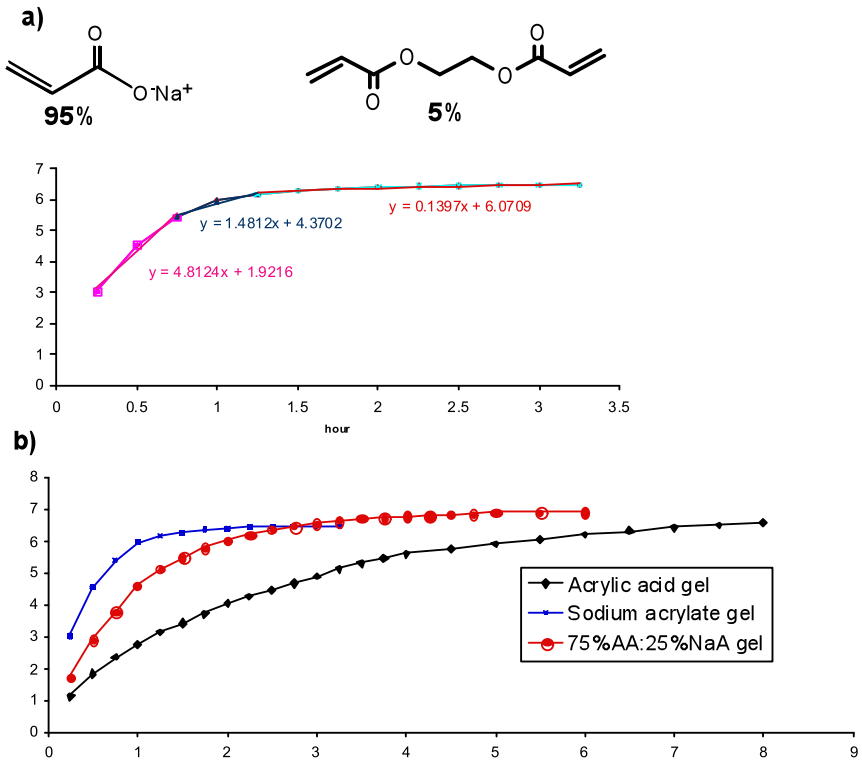

Figure 2. a) Release profile of Morphine. b) Release of Morphine from the gels prepared from acrylic acid and sodium acrylate monomers.

Moreover, to be able to tune the release of morphine, we prepared gels by chemically mixing the monomers, acrylic acid, and sodium acrylate. We indeed found that gels formed from the co-mixture of these two monomers showed tunable release kinetics compared to that of gels prepared either from acrylic acid or sodium acrylate (Figure 2b). We were also interested in physically mixing the acrylic acid and sodium acrylate based gels to see the how it affects the morphine release. We obtained a burst release, followed by the slow release of morphine from the gels.

Design and Synthesis of Acetal Cross-linked CO_2-Responsive Gels

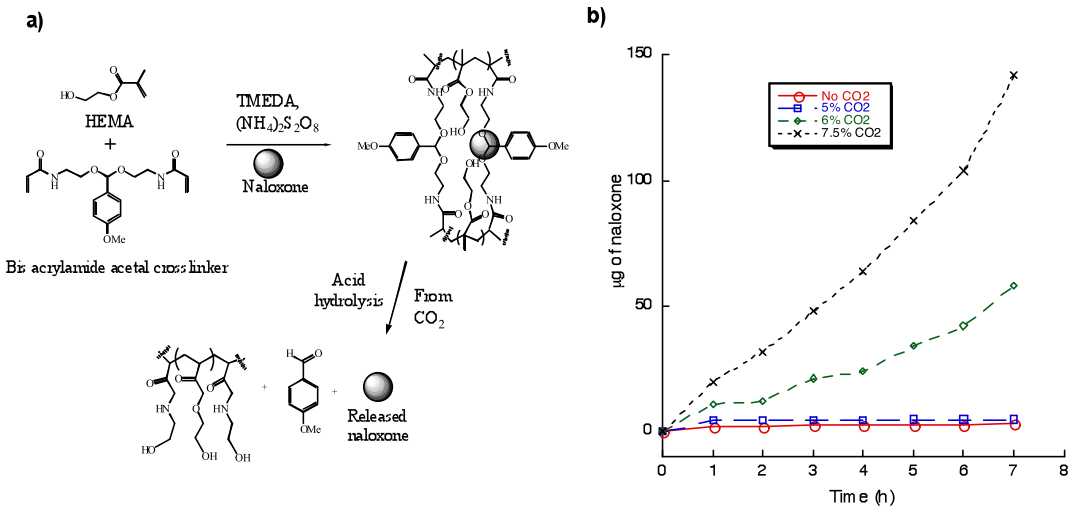

Figure 3. a) Synthetic scheme for acetal-cross-linked hydrogels. b) Release profile of Naloxone in response to CO_2

We were also interested in releasing an antidote, Naloxone, from hydrogels in response to CO_2. For CO_2 responsive functional group, we targeted acetals which are known to respond to changes in the pH. We prepared gels from HEMA and acetal-based cross linker in the presence of Naloxone (Figure 3a). The resultant gel was then subjected to different percentage of CO_2 to study the release of Naloxone from the gel. We found that there is a clear dependence in release rate of Naloxone in response to $\%CO_2$. There was essentially no release with 5% CO_2 whereas 7.5% CO_2 resulted in significant amount of Naloxone release from the acetal-cross linked gels (Figure 3b).

Toxicity Studies

Since the acetal cross-linked gel showed promising results for Naloxone release in response to CO_2 we were interested in studying the toxicity of these gels. Toxicity was tested up to 48 h and acetal-cross linked gels displayed no toxicity (Figure 4). We also found that degradation products did not exhibit any toxicity.

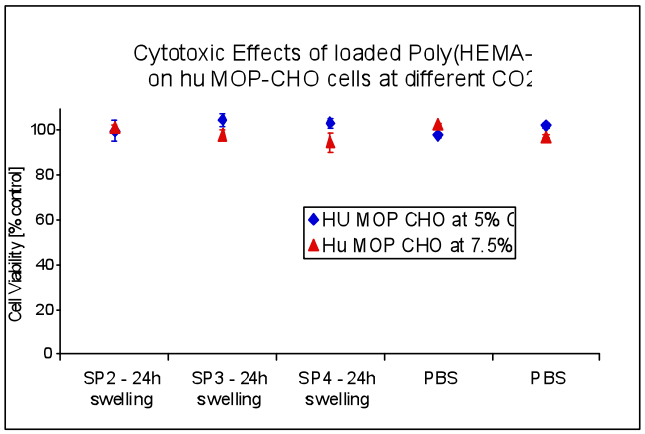

Figure 4. Cytotoxicity of loaded acetal cross-linked gels

2008-09

In this project year, we specifically aimed at the design and synthesis of polymer hydrogels for morphine and naloxone release in response to biomarker, CO_2.

Design and Synthesis of Cationic Hydrogels Responsive to CO_2

Taking advantage of CO_2 as a toxicity marker, a hydrogel-based delivery vehicle containing dimethylamino groups [poly(N,N-dimethylaminoethyl methacrylate) cross-linked by trimethylolpropane tri-methacrylate] was designed and synthesized (Figure 1).

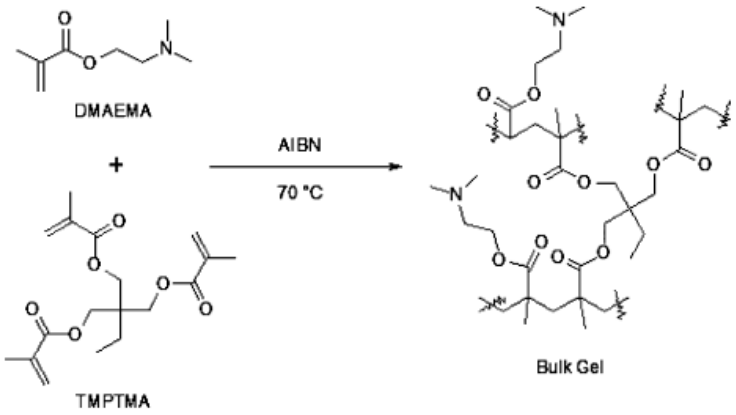

Figure 1. Synthetic scheme for CO_2 responsive cationic gel.

We first studied the release of Naloxone from these cationic hydrogels in response to CO_2. Among all the compositions studied hydrogels of poly-(DMAEMA/TMPTMA) with 90:10 showed a stronger response to CO_2 compared to 85:15 or 80:20 (Figure 2). The increased cross-linking density reduced the swelling of the gel and thus controlled the release of drugs. Moreover, the increase in cross-linking density causes a simultaneous decrease in the content of the functional groups. Therefore, a right combination of percentage DMAEMA and the TMPTMA are necessary to effectively control the release of drugs over time, in addition to the concentration of he stimulus. It appears that, at higher cross-linking density (>20%), the loading of drugs occurs through surface adsorption and thus results in a higher percent drug release independent of the concentration of the stimulus, as evidenced for 80:20 DMAEMA/TMPTMA gel (Figure 2).

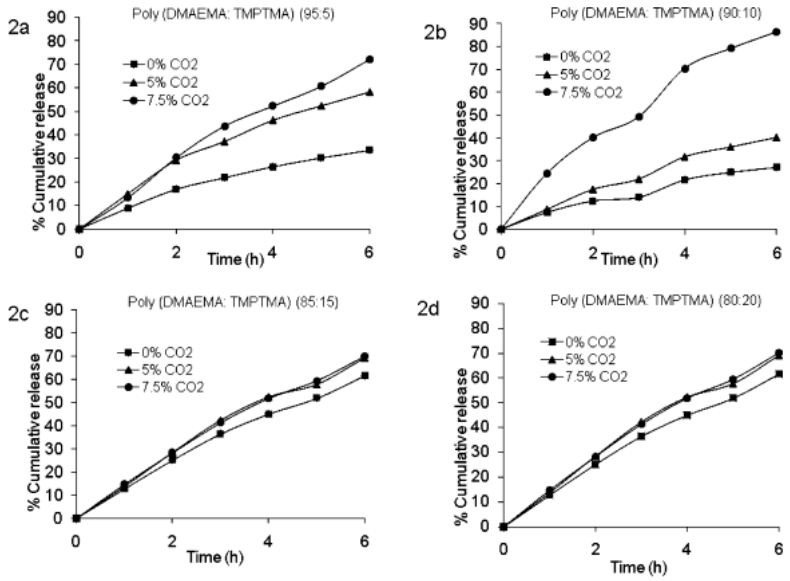

Figure 2. Release of Naloxone from hydrogels with varying cross-linking densities.

The DMAEMA gels are sensitive to pH as well. Because change in CO_2 concentrations can cause subtle changes in pH, we were interested in investigating the pH-dependent release profile and comparing these with the CO_2 -dependent release profiles above. Naloxone release from the hydrogels was studied by dispersing drug-loaded poly(DMAEMA/TMPTMA, 90:10) in buffered solutions, at a specific pH. The cumulative percent release of naloxone and percent swelling of the gels after 6 h are listed in Table 1. The naloxone release rate is directly affected by the pH of the release medium. Reduction of the pH from 7.4 to 6.8 increased the swelling of the gel by about 47% and thus increased percent drug release from 37 to 89%, respectively. The results obtained from pH responsive naloxone release were correlated with that of CO_2. We were gratified to note that the cumulative naloxone release at 5 and 7.5% CO_2 (40 and 86%) compared well with the corresponding release at pH 7.4 and 6.8 (37 and 89%).

release medium	% swelling	% naloxone released
pH 7.4	104	37
pH 7.2	112	58
pH 7.0	128	78
pH 6.8	151	89
0% CO_2 (pH 7.7)	94	27
5% CO_2	110	40
7.5% CO_2	127	86

Table 1. Percentage Swelling of DMAEMA/TMPTMA (90:10) Gels and Percentage Naloxone Released after 6 h

2009-10

During this project year, we were particularly interested in the design and synthesis of feedback-responsive nanogels for intravenous injection.

Design and Synthesis of Feedback-Responsive Nanogels for Intravenous Injection

So far, we have discussed the design and synthesis of bulk gels which are capable of releasing Naloxone in response to CO_2. However, it is highly desirable to design chemically cross-linked, highly stable, water soluble polymeric gels because water soluble nanogels can be intravenously administrated. Therefore, we were interested in designing nanogels responsive to feedback provided by the body. We developed a facile method for the preparation of nanogels with hydrophobic guest encapsulation capabilities. Herein, we mainly focused on;

i) Development of an emulsion free method for stable nanogel synthesis
ii) Studying the container ability of the nanogels synthesized
iii) Disassembly of the nanogels in response to specific feedback provided by the body.
iv) Testing the stability of nanogels

Emulsion-Free Nanogel Synthesis and Disassembly of Nanogels in the Presence of Feedback Provided by the Body

Chemically cross-linked, water-soluble polymer nanoparticles constitute a promising scaffold in therapeutic delivery applications, offering potential to circumvent stability issues. However, these polymeric nanoparticles or nanogels face certain complications, as they are prepared by microemulsion or inverse microemulsion methods. These methods are relatively complex and require multiple purification steps to remove not only unreacted monomer but also the surfactant materials that were used to stabilize the emulsion. When a water-soluble polymer nanoparticle is desired, inverse microemulsion based synthesis is the preferable method. Note that the continuous phase in the inverse microemulsion (water-in-oil emulsion) method is based on a lipophilic solvent and therefore cannot be used to encapsulate hydrophobic guest molecules during nanoparticle formation. This is a significant limitation for feedback regulated drug delivery applications because most of the drug molecules are hydrophobic. Therefore, we were interested in developing an emulsion-free method which has the following features; *(i)* guest molecules can be easily incorporated noncovalently within the nanoparticles; *(ii)*

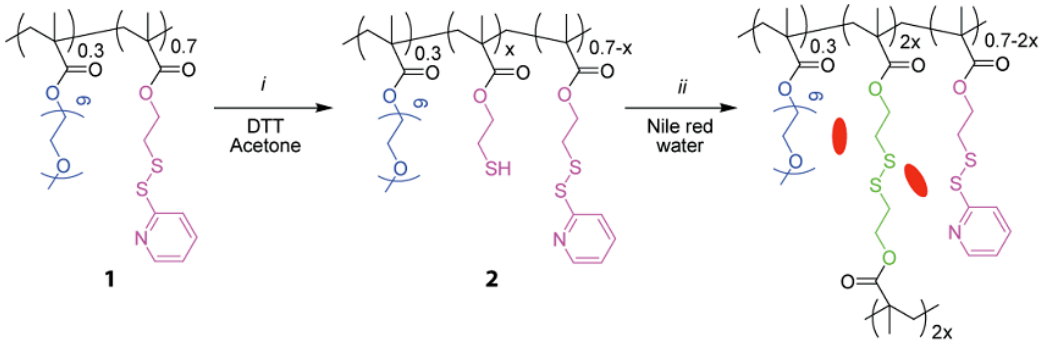

Figure 1. Synthetic scheme for emulsion-free nanogel synthesis.

the noncovalently encapsulated guest molecules can be released in response to a biologically relevant feedback.

We have shown that addition of a deficient amount of dithiothreitol (DTT) to random polymer, **1** where the hydrophobic block is composed of pyridyldisulfide (PDS) groups converted the corresponding small percentage of PDS groups to free thiols, represented by the structure **2** in Figure 1. These free thiols then reacted with an equivalent amount of the remaining PDS functionalities to create disulfide bonds, which effectively cross-linked the polymer chains. We also demonstrated that the hydrophobic interior in the aggregate provided an opportunity to encapsulate lipophilic guest molecules prior to cross-linking. Note that for biomarker-responsive functional group in our initial studies, we targeted a disulfide bond, since these bonds are susceptible to biochemical feedback such as glutathione (GSH).

Next, to investigate the possibility of encapsulating a hydrophobic guest molecule within the interiors of these nanogels, we carried out the DTT-based cross-linking reaction in the presence of Nile red, a hydrophobic dye and then release of the encapsulated Nile red was demonstrated in the presence of biologically relevant biomarker GSH. We also demonstrated that guest molecule release can be tuned by varying the cross-linking density of these nanogels. For example, we prepared 6 (NG1), 13 (NG2), and 25% (NG3) cross-linked nanogels and we demonstrated the tunability of guest release from these nanogels in the presence of 10 mM GSH (Figure 2).

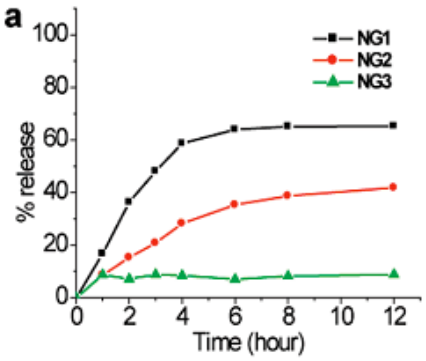

Figure 2. Comparison of GSH-induced dye release rate from the nanogels, which have different cross-linking densities at pH 7.4

We anticipated that our gels would be relatively nontoxic, because they are made from biocompatible oligoethyleneglycol components as surface displays in a methacrylate backbone. The nanogels indeed exhibit high cell viability and no concentration-dependent toxicity up to a nanogel concentration of 1 mg/mL. This result indicates that the nanogel material is nontoxic and thus a potential candidate for biological applications.

Non-covalent Encapsulation Studies

Encapsulation stability plays a crucial role in supramolecular assemblies as it prevents the premature release of payload before approaching the target site. Thus, an analysis of this process is necessary for optimization of the design and construction of drug delivery carriers. Therefore, we were interested in developing a simple method to test the stability of our nanogels we synthesized using emulsion-free method developed by our group. Our method involves the measurement of dynamics of guest interchange in nanocarriers using Fluorescence Resonance Energy Transfer (FRET) as a tool and compares it with that observed in classical amphiphilic nanoassemblies. A lipophilic

FRET pair, (DiO, donor) and (DiI, acceptor) is used for this purpose. These two dye molecules are independently sequestered in our nanogels with varying cross-linking densities. When the solutions containing the dye molecules are mixed, two limiting scenarios are possible (Figure 3a). If the dye molecules are stably encapsulated and do not exchange the guest molecules with the bulk solvent environment, then the two dye molecules will continue to be in two separate nanocontainers. If this were the case, no FRET would be observed since the distance between the two dye molecules is much higher than their Forster radius. However, if there is a significant exchange of the guest molecules between the container interior and the bulk solvent when the two solutions are mixed, it is likely that the dye molecules will equilibrate between the two containers. This is because there would be no registry regarding the nanocontainer from which the dye molecule leaked. The resulting equilibration will cause DiI and DiO molecules to occupy the same container, leading to increased FRET.

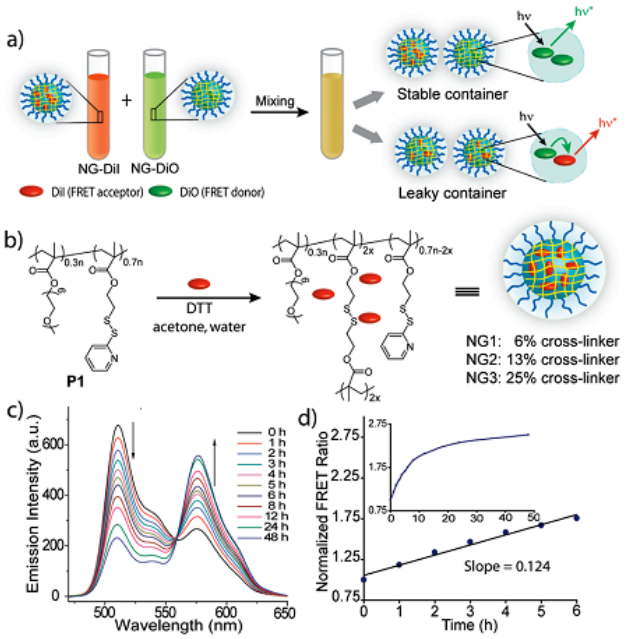

Figure 3. a) Mixed nanogels encapsulated DiI/DiO and FRET behavior.

We first tested our method using 6% cross-linked nanogels. The two solutions containing the separate dyes, referred as NG1-DiO and NG1-DiI, were then mixed in water. Fluorescence from the DiO excitation (450 nm) was monitored over time. The evolution of FRET was obtained by tracing the decrease in the donor (DiO) emission and concurrent increase in the acceptor's emission (DiI). The results show that there is a gradual equilibration of the dye molecules over a 48 h period (Figure 3c). The FRET ratio was plotted against time (Figure 3d). The slope of the linear fit is related to the dynamics of the guest exchange, and we define this as the leakage coefficient (Λ), which was found to be $\sim$0.124 h^{-1} for the first 6 h in NG1 (Figure 3d). We envisaged that cross-linking density could be used to tune the rate of exchange/leakage. The preparation method indeed allows for control over the degree of cross-linking. NG2 and NG3, 13% and 25% cross-linked, exhibited minimal exchange over 6 h at Λ of 0.002 h^{-1} or below, compared to 0.124 h^{-1} for NG1. These results suggest that the degree of cross-linking is effective in tuning guest exchange dynamics. In other words, we find that the guest exchange is slower in cross-linked polymer nanogels and that this can be conveniently tuned by altering the degree of cross-linking.

2010-11

We have previously developed a class of versatile nanogels that will be useful in intravenoud administration of drug molecules, which has similar biodistribution as the targeted intraosseous adminstration. During this project year, we were particularly interested in the design and synthesis of CO2-responsive (biomarker for opioid-induced toxicity) nanogels for intravenous injection.

Design and synthesis of acetal-cross-linked CO2 responsive nanogels.

Compared with bulk gels, one of key advantages of nanogels is that they can be intravenously injected. If we can incorporate CO2 responsive acetal crosslinkers into nanogels, after intravenous administration, the release of payload could be regulated by the real-time CO2 concentration in blood. To prepare CO2 responsive nanogels, a suitable synthetic strategy is highly demanded. In addition to preparing nanogels from PDS containing amphiphilic copolymers, we also developed a new methodology to synthesize nanogels from pentafluorophenyl-activated acrylate containing random copolymers cross-linked by diamine cross-linkers (Figure 1). The post-nanogel modification makes it possible to remove the potentially toxic pentafluorophenyl groups and incorporate different functionalities onto nanogel surface.

Using the same strategy, CO2 responsive nanogels loaded with guest molecules were obtained via reaction of amino acetal cross-linkers toward PFP activated ester. The cross-linking was monitored by FTIR and ^{19}FNMR respectively. To investigate how CO2 triggers the release of payload from nanogels, a FRET technique developed to measure the dynamics of guest interchange was employed. In particularly, the FRET

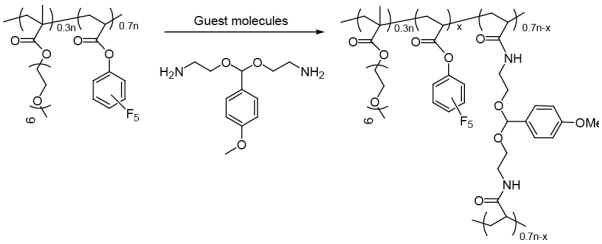

Figure 2. Synthesis of acetal cross-linked nanogels

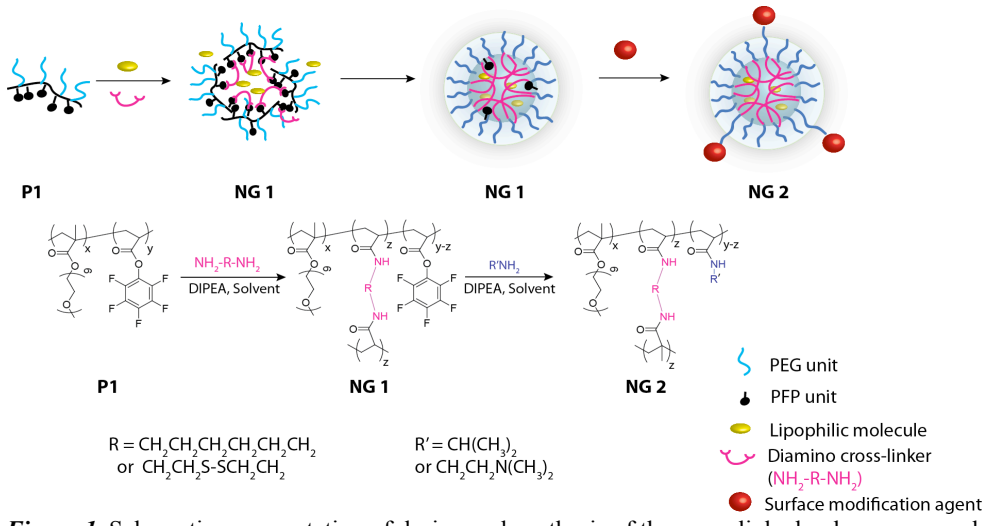

Figure 1. Schematic representation of design and synthesis of the cross-linked polymer nanogels

evolution was measured after nanogels independently encapsulated with DiI (FRET

acceptor) and DiO (FRET donor) were mixed together and treated with CO2 bubbling. Figure 3 shows the FRET pattern of mixed nanogels which were 20% cross-linked with respect to PFP. Encapsulation of guest molecules without stimuli in both nanogels and polymer precursors are very stable indicated by the lack of observation of FRET evolution even in 11 hours. As soon as nanogels and polymers were treated with 7.5% CO2, FRET evolutions were observed immediately in both cases. However, guest interchanges in nanogels are much faster than what happens in polymers, which can be quantified by FRET ratio defined as $I_a/(I_a+I_b)$. The calculated FRET ratios shown in Figure 3f clearly suggests that guest interchanges in nanogels are highly enhanced by CO2 purging. Accelerated dynamics of guest interchanges is also observed in polymers when purged with 7.5% CO2 though the acceleration is much less than that in nanogels. The unexpected increase on guest interchanges in polymers is probably the result of physical enhancement on surface binding guest molecules interchanging due to bubbling. If the hypothesis is right, similar enhanced FRET evolution will be observed when nanogels are purged with other gas rather than CO2 that supposed not to breakdown cross-linkers. To test this hypothesis, the FRET behavior was followed when we purged mixed nanogels with argon (Figure 3c). Interestingly, compared with polymers treated with CO2, FRET was enhanced to a similar extent when nanogels were subjected the argon purging. These results suggest nanogels prepared from pentafluorophenyl activated polymer cross-linked by acetyl are CO2 responsive.

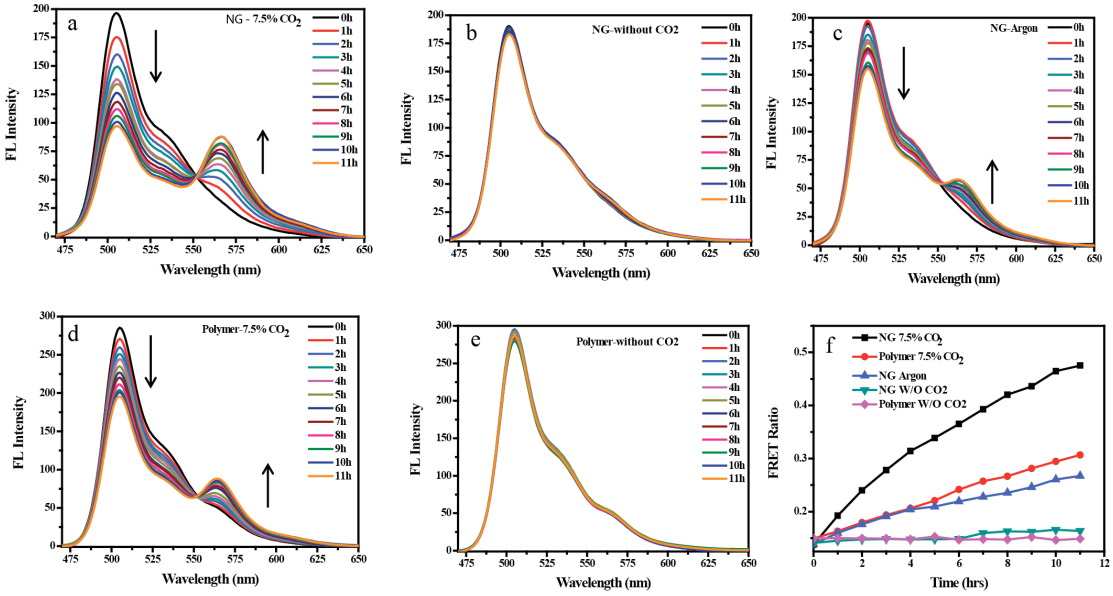

Figure 3. Dynamics of guest molecules interchange: a, b, d and e are nanogels and polymers purged with and without 7.5% CO2 air, respectively; c is nanogels purged with Argon. f is the FRET ratios calculated from a,b,c,d and e.

A potential issue might hinder the drug delivery application of these nanogels is the toxicity to remaining pentafluorophenyl groups. To address this problem, we are interested in removing remained PFP groups after cross-linking via post-nanogel

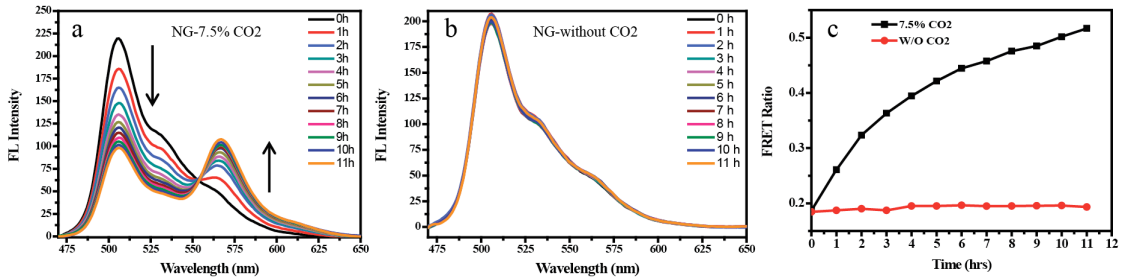

Figure 4. Guest interchanging in octylamine substituted nanogels; a: nanogels treated with 7.5% CO2; b: without CO2 purging; c: FRET ratio calculated from a and b. Nanogels are 20% cross-linked and remaining PFPs are fully replaced by octylamine.

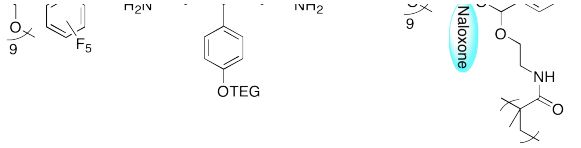

Figure 5. Representation of preparation of naloxone loaded nanogels

substitution. One of the key prerequisite of post-nanogel substitution is not to impair the encapsulation stability too much. The same FRET based technique was employed to investigate the encapsulation stability of nanogels in which all the remained PFP groups were fully replaced by octylamine after cross-linking. No FRET evolution in 11 hours indicates nanogles encapsulation stability after encapsulation is still very high. Also, nanogels respond to CO2 purging in a similar fashion to their unsubstituted counterparts.

In Vivo study

We are also interested in *in vivo* application of these CO2-responsive nanogels in delivery of naloxone. A variety of nanogels with three cross-linking densities (10%, 30% and50%) prepared from two polymer precursors with various PEGMA/PFPMA ratios (1:1, 4:6) are shown in Figure 5. Nanogels are denoted indicating the composition of precursor polymer and cross-linking density. For instance, 1:1-10 means nanogels were prepared from polymer with a PFGMA/PFPMA ratio of 1:1 and 10% cross-linked. Theoretical naloxone loading of all nanogels is 10 wt%. Respiratory depression as shown in Figure 6 was followed by monitoring the Minute Ventilation after mice were intravenously injected nanogels. Here, Minute Ventilation were measured by taking the animal from room air to hypercapnia air (5% CO2) and see how it responds to that environmental change. The breathing change in normal air condition is defined as 100%. When mice are sedated, the change is

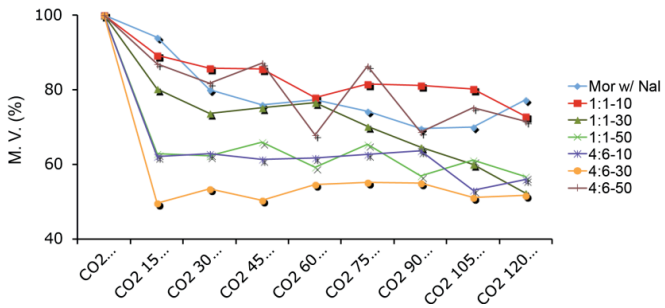

Figure 6. Breathing change of animals injected with nanogels

typically less than 100% and this is quantified. So, when the M/V is smaller, they are more depressed. Then naloxone injected animals are all safely depressed without receiving life-threatening rescue injection. Additionally, with 1:1 nanogels, minute ventilation is cross-linking density dependent.

Table 1. Naloxone blood concentration at 2 hours in respiratory animals

Animal	Polymer	Naloxone (mg/kg)	Naloxone Conc @ 2 h (ng/ml)
167	1:1-10	0.8	51.2
168	4:6-50	0.8	58.7
169	4:6-10	0.8	89.7
170	1:1-30	0.4	24.1
171	4:6-30 dil	0.2	23.7
172	4:6-50 dil	0.2	21.3
173	4:6-10 dil	0.2	50.4
174	1:1-50	0.8	83.7
176	4:6-30	0.8	151.7
6 Animals	Free Naloxone	1.0	7.2

Pharmacokinetic was also investigated. The concentration of naloxone in blood was measured after respiratory depression analysis and shown in Table 1. PK data sugguest that: i) Naloxone administered through the nanogels is detectable in the blood for longer at higher concentrations; ii) the nanogels release naloxone over time and protect it from metabolism while inside the nanogels. Morphine and naloxone blood concentrations are also detected independently shown in Figure 7. The similar evolution of morphine blood concentrations in mice injected with free naloxone/morphine and naloxone loaded nanogel/morphine suggests the presence of naloxone nanogel does not effect the metabolism of morphine. Naloxone concentration was higher than injection with free naloxone and morphine when animal was injected with naloxone nanogel and morphine.

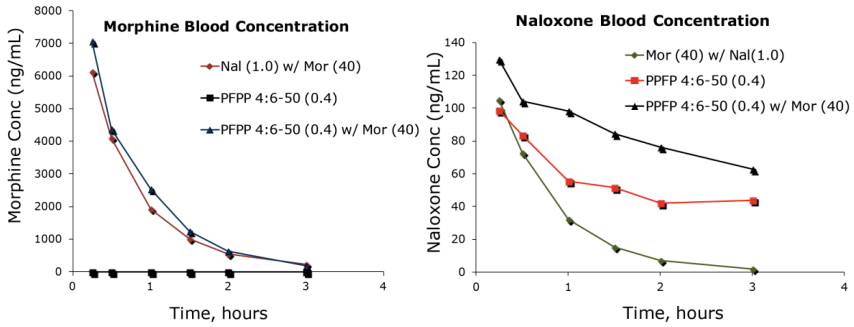

Figure 7. Morphine and Naloxone blood concentration

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